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Effects of a high-protein, low-energy diet in finishing lambs: 1. Feed intake, estimated nutrient uptake, and levels of plasma metabolites and metabolic hormones

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Abstract

Patterns of tissue mobilisation in ruminants are ill-understood. This hinders nutritional management to mitigate the effects of energy deprivation on protein mass, and nutritional regimens designed to change body composition. An experiment was conducted to comprehensively evaluate the effects of a low-energy high-protein diet in growing lambs. Three diets (CON = concentrate diet ad libitum, STR = straw ad libitum, SFM = straw ad libitum plus 150 g/d fish meal) were fed to growing white-face lambs. Analysis of feed intake and metabolite data provided evidence that greater available N did not have a synergistic effect on intake of low quality forage. Metabolite and hormone profiles of lambs in negative energy balance, supplemented with duodenally available protein, revealed that SFM animals did not respond with an accelerated rate of fat mobilization or maintain protein mass due to available N. This was particularly evident from the leptin profiles, which indicated higher circulating leptin levels for SFM compared to STR animals. Further, the data revealed that in sheep fed below requirements for maintenance, leptin levels did not correspond with acute ME intake, whereas the opposite was true for well-fed animals. Conversely, the response of the GH/IGF-1 axis to high protein–energy ratio (PER) diets was indicative of effects not explained by the difference in energy provided by the two experimental diets.

These results contrast with observations from intra-gastric infusion experiments testing similar PERs and previous conventional feeding trials and provide evidence of: (1) specific differences associated with PER in lambs fed via intra-gastric infusion vs. a conventional feeding approach, and (2) lack of usefulness of high PER diets in the post hoc modification of body composition of growing lambs. While positive N balance is known to occur in ruminants in negative energy balance, the determinants of relative proportions of muscle and adipose tissue catabolised under energy deprivation remain unknown.

Keywords: Sheep; Protein–energy ratio; Body composition; Catabolism; Leptin

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1. Introduction

Interest in increased production efficiency and concerns about overfat meat animals motivated significant research efforts into pharmacological interventions directed at the modification of body composition of meat animals. However, regulatory and consumer resistance, especially in Europe, have prevented their introduction into production systems. Nonetheless, the degree of fatness of meat animals is among the most important concerns of consumers, and so there is continued interest in nutritional regimes that could alter body composition without affecting carcass value. Thus, effects of protein–energy ratio (PER) in the diet of ruminants on energy and N balance and body composition have received considerable attention; even though they are difficult to study, due to the extensive transformation that nutrients undergo in the forestomachs. The problem has important ramifications in several areas, including: understanding of the interdependence of energy and protein metabolism, understanding of the regulation of tissue catabolism, and the need to develop feeding regimes that optimise a balance of protein and energy supply.

Ørskov et al. (1979) and Hovell et al. (1983) demonstrated that high PERs supplied by an intra-gastric infusion regimen caused sheep to attain positive N balance while in negative energy balance. Chowdhury et al. (1990) reported similar results for cattle. These data suggested that endogenous fuel reserves (fat depots in particular), could serve to sustain lean tissue accretion in situations where feed energy is limiting, provided that ruminants were supplied with tissue-available protein. Tamminga et al. (1997) reported that conventionally fed dairy cows regularly reach positive N balance while mobilising substantial quantities of adipose tissue in early lactation. However, experiments with sheep utilising conventional feeding approaches have produced results not always in agreement with expectations generated by intra-gastric infusion experiments or observations from catabolic dairy cows. Fattet et al. (1984) found that lambs given NaOH-treated straw supplemented with fish meal had a positive change in body protein while concomitantly losing body fat. Similarly, Vipond et al. (1989) found significantly more saleable lean from lambs supplemented with fish meal vs. non-supplemented lambs on a straw diet. However, both research teams found fat loss to be either equal or lower in the N supplemented groups. These observations do not support an enhanced use of endogenous fuels for protein accretion under high N supply. In a similar experiment, Galbraith et al. (1997) did not find enhanced protein accretion in lambs, but rather a tendency toward lower peripheral fat mass and higher carcass fat losses for N supplemented growing lambs, compared to unsupplemented lambs.

In a review by Chowdhury and Ørskov (1997) the discordance in response to intra-gastric infusion vs. conventional feeding regimens (at comparable PERs) was not addressed. Several factors may contribute to such conflicting results, among them high between-animal variability, different stage of maturity of experimental animals, and differences between the PER intended vs. that effectively achieved. A comparison of data within the literature is therefore made difficult by differences in experimental protocols, type and age of animals, and duration of regimens. The objective of this study was to conduct a comprehensive evaluation of the effects of a high-protein/low-energy diet in growing lambs. We intended to evaluate the hypothesis that supplementation with rumen-degradation resistant protein causes accelerated breakdown of adipose tissues and conservation of proteic tissues under energy deprivation in sheep. In this paper we report the effects of high protein supplementation under energy deprivation on metabolite and hormone profiles and estimated nutrient intake. In a subsequent paper we report the effects of this nutritional regimen on weight change, organ mass, body composition, fatty acid composition of lean and adipose tissue, and taste panel evaluation.

2. Materials and methods

2.1. Animals, feeding

All experimental procedures were carried out at the U.S. Sheep Experiment Station, Dubois, ID, with approval of the Institutional Animal Care and Use Committee. Eighty-one growing white-face wethers (representative of typical commercial range sheep in the western U.S.) were fed ad libitum after weaning a concentrate/alfalfa pellet diet, supplemented with a

commercial concentrate pellet, to an average weight of 56.6 ± 4.6 kg. Lambs were then randomly assigned to one of three nutritional treatments: 1) Control (CON, $N=25$), continued on the feedlot diet ad libitum, 2) straw–fish meal (SFM, $N=28$) with barley straw ad libitum supplemented with 75 g/d (as fed) molasses and 150 g/d (as fed) fish meal, and 3) straw supplemented with 75 g/d (as fed) molasses (STR, $N=28$). Lambs assigned to SFM and STR were adapted to the straw diet over a period of one week by gradually replacing the adaptation diet with straw. Lambs on diet SFM did not receive fish meal during the adaptation period, reproducing the design of previous studies (Vipond et al., 1989; Fattet et al., 1984). During the adaptation phase of the study, STR and SFM lambs lost weight while CON lambs continued to gain weight. Average weight of lambs at the start of the experimental treatment phase was estimated by interpolating between weighing dates, bracketing the final diet change. The on-treatment live weights were: 55.6 ± 3.9, 53.3 ± 3.6, and 59.5 ± 3.3 kg for treatments SFM, STR, and CON, respectively. At the beginning of the experimental treatment phase, lambs were approximately 10 months old. A total of 54 lambs (selected at random within dietary treatment groups, 18 per group) were serially slaughtered over the 5 weeks of study to assess the impact of diets on body composition and carcass traits.

2.2. Animal housing and management

All lambs were dewormed prior to study and housed indoors in group pens with concrete floors. Pens contained 3 to 4 lambs/pen and were equipped with slatted wooden pedestals. Artificial light with a 12/12 h regime was provided. Lambs were fed between 7 and 8 a.m. Straw was given first, then top-dressed with a molasses–fish meal slurry. Feed bunks were refilled with straw in the afternoon. CON lambs were fed only once per day. Water was available ad libitum and a salt-trace mineral mixture was provided as required.

2.3. Measurements

Lambs were weighed once weekly before feeding. Feed refusals were collected and weighed daily, thoroughly mixed and composited weekly per pen. Blood samples were collected twice weekly by jugular venipuncture into sterile tubes containing 0.117 ml of 15% EDTA (K_3) prior to feeding for the determination of metabolites and hormones. Samples were centrifuged at 3500 $\times g$ for 20 min; plasma was harvested and stored frozen at −20 °C for later analysis. The sample set closest in time to weighing (5 samples spaced one week apart) was analysed for circulating metabolite levels. The first of these samples was taken shortly after the start of the one-week feed adaptation period; consequently, it is considered a baseline measurement. All available blood samples (10 sampling dates total) were assayed for leptin, growth hormone, and IGF-1.

2.4. Metabolite analyses

Plasma samples were analysed using enzymatic, colorimetric procedures for non-esterified fatty acids (Wako Kit #994-75409E, Wako Chemical Company, Richmond, VA) and beta-hydroxybutyrate (Sigma Kit #310-A, Sigma Chemical Company, St. Louis, MO). Analysis for blood urea nitrogen (BUN) and glucose (GLU) was conducted using slide chemistry on a Vitros DT-60 Blood Analyzer (Johnson and Johnson Clinical Diagnostics, Rochester, NY).

2.5. Hormone assays

Plasma samples were analysed for leptin, GH, and IGF-I. Leptin is mostly synthesized in adipose tissue, while GH and IGF-1 are intimately related to the regulation of growth. Each hormone assay determination was performed in triplicate via double antibody radioimmunoassays previously validated within our laboratory (Delavaud et al., 2000; Powell and Keisler, 1995; Morrison et al., 2002). Inter and intra-assay coefficients of variation were less than 8%. Samples for all 10 collection dates (2 per week) were analysed.

2.6. Analyses of feeds and refusals

Dry matter was determined by drying feeds and refusals at 100 °C for 8 h. Organic matter and ash were measured by burning the sample at 620 °C in a muffle furnace for 4 h. Dried feed and refusal samples were ground to pass a 1-mm screen in a UDY Cyclone

mill. Samples were analysed for DM, ash, Kjeldahl N (crude protein, CP) (AOAC, 1984), and in vitro dry matter digestibility (IVDMD; Tilley and Terry, 1963). In vitro true digestibility was determined using a DAISY^{II} Incubator (ANKOM Technology, Fairport, NY). Rumen fluid was obtained from sheep fed a low quality hay–straw mixture for determination of IVDMD of straw from treatment STR. For analysis of straw IVDMD for treatment SFM, donor fluid was obtained from ewes fed a high-quality alfalfa hay/alfalfa pellet mixture.

2.7. Data preparation and statistical analyses

2.7.1. Feed intake

Feed intake data were compiled and analysed on a per pen basis. Since all pens were identical and environmental conditions were controlled, no systematic pen effect was expected. Pen assignments were changed during the course of the experiment for handling purposes arising from the need to maintain minimum group sizes after removal of lambs for serial slaughter. These changes were carefully planned in order to avoid confounding of the analysis of intake and refusal data, which was based on pen as the experimental unit. The following linear model was used: $y_{ij}=\mu+\tau_i+\varepsilon_{(i)j}$ where y_{ij} denotes the average feed intake per jth animal within pen, and τ represents the fixed effect of the ith treatment with three levels. Additionally, quantity of fish meal offered to the groups under treatment SFM was subtracted from observed intake in order to identify possible interactive effects of fish meal on consumption of straw. The resultant quantity 'SFM minus FM' was included as the additional factor level ST − FM.

2.7.2. Refusals

CP and ash content data of refusal samples (with pen as experimental unit) were analysed with linear models to determine systematic differences between weeks that might be relevant for the interpretation of metabolite and body composition data. For the complete refusal data set, the following model was used: $y_{ijk}=\mu+\tau_i+\delta_j+\tau\delta_{ij}+\varepsilon_{(ij)k}$ where τ is the effect of the ith treatment level, δ is the effect of the jth date, and $\tau\delta$ is the main effect interaction. All treatments were further analysed individually for the effect of date only. All response data were transformed with the arcsine-square root transformation to approximate normality.

2.7.3. Metabolites and hormones

Metabolite and hormone data (with individual animal as the experimental unit) were analysed with the following linear repeated measures model: $y_{ijk}=\mu+\tau_i+\delta_j+(\tau\delta)_{ij}+\varepsilon_{(ij)k}$ where τ is the effect of the ith treatment level, δ is the effect of jth collection day, $\tau\delta$ is the interaction between ith treatment level and jth collection day, and is the random experimental error associated with the kth animal assigned to the ith treatment at the jth collection day. This mean model was run with all available covariance structures for the within-subject measurements. Selection of the most appropriate covariance structure was based on Akaike's Information Criterion (SAS, 1999). Fixed effects were estimated with the final model selected. Treatment effects were affected by interaction with the within-animal factor time. Accordingly, specific contrasts were estimated with sliced effect evaluation (SAS, 1999) for each sampling date. Further, the statistical significance of treatment differences in changes between levels measured on the first collection and the last collection was estimated for certain treatments. Significance tests for these multiple comparisons were adjusted by the Tukey–Kramer adjustment. Although this adjustment is not maximally conservative for the control of experiment-wise error rate, it provides guidance in interpreting differences between treatment mean curves. All statistical analyses were performed with SAS System Version 8.2 (SAS Institute, Inc. 1999). Where needed, data were transformed to approximate normality. Significance of mean separation tests was declared at $p=0.05$.

2.7.4. Estimated energy and protein uptake

Average energy intake (with pen as experimental unit) was estimated using two methods: (1) based on tabled values of ME content of feedstuffs used in the diets (NRC, 1985), and (2) based on IVDMD results (Table 1). ME intake from fish meal was corrected for estimated effective intake of fish meal based on proximate analysis of refusals. During the first week of experimental treatments, CP values obtained for refusals from SFM pens indicated that a substantial amount of fish meal had not been consumed. For subsequent weeks, the average difference in CP

Table 1
Diets, ingredients and proximate analysis results

Diet	Ingredients	% DM	% OM	% Ash	% CP
Control (CON)	Corn[a] (65%, w/w)	88.2	99.76	0.24	6.55
	Alfalfa pellet (25%, w/w)	88.5	90.09	9.91	15.88
	CTC[b] 100 (5%, w/w)	90.9	92.39	7.61	15.24
	CTC[b] 300 (5%, w/w)	91.74	83.06	16.94	36.41
Straw–fish meal (SFM)	Straw[a] (ad lib)	93.4	91.17	8.83	2.05
	Molasses (75 g/d)	47.76	92.36	7.64	10.9
	Fish meal (150 g/d)	90.42	80.45	19.55	69.25
Straw (STR)	Straw[c] (ad lib)	93.4	91.17	8.83	2.05
	Molasses[c] (75 g/d)	47.76	92.36	7.64	10.9

[a] Mean of two approx. equal quantities fed.
[b] Commercial supplement.
[c] Ingredients were the same as for diet SFM.

content of refusals of treatments STR and SFM was small (Table 2). The differences in CP content of refusals within treatment SFM were not significant between weeks 2, 3 and 4, and did not differ from the average of treatment STR, indicating complete consumption of fish meal after week 2.

Daily N flux in the duodenum from ruminal digestion of straw for diets SFM and STR, and for digestion of diet CON was estimated with the following equation: CP=1.3*6.25 (g/MJ ME) where ME denotes intake of metabolizable energy. This is a modification of the ARC equation for yield of microbial protein (ARC, 1984). The lower coefficient was chosen because, as noted by ARC, the original equation is applicable to well-balanced roughage/concentrate diets. Neither of the diets used in the present study falls into that category. This coefficient was further reduced to 1.1 for the STR diet treatment, because, as indicated via in vitro digestibility data, there was a deficiency of available N sufficient to maintain microbial protein production at levels comparable to treatments SFM or CON. In order to illustrate the magnitude of the effects of tabulated vs. in vitro digestibility data, microbial protein yield was calculated for ME intake based on both. Values for undegraded CP for the ingredients of diets CON and STR were not available; however, the assumption that the contribution of undegraded CP to duodenal flux

Table 2
Proximate and statistical analysis of feed refusals

Diet	Refusals	% DM	% OM	% Ash	% CP
Control (CON)	Week 1	90.77b ± 0.28	98.58 ± 0.921	1.423 ± 0.92	8.11 ± 1.96
	Week 2	90.43 ± 0.04	98.72 ± 0.145	1.28 ± 0.145	8.72 ± 0.44
	Week 3	89.73 ± 0.1	98.81 ± 0.566	1.19 ± 0.566	9.1 ± 0.93
	Week 4[a]	90.73	96.21	3.79	13.24
Straw–fish meal (SFM)	Week 1	93.48 ± 0.47	82.07 ± 2.92	17.93 ± 2.92	32.1 ± 9.7[b]
	Week 2	94.4 ± 0.47	90.92 ± 0.513	9.08 ± 0.513	1.91 ± 0.177[c]
	Week 3	93.96 ± 0.16	91.86 ± 0.272	8.14 ± 0.272	1.73 ± 0.09[c]
	Week 4	94.54 ± 0.42	91.51 ± 0.421	8.49 ± 0.42	3.46 ± 1.19[c]
Straw (STR)	Week 1	93.85 ± 0.52	89.7 ± 1.44	10.33 ± 1.44	1.85 ± 0.45[b]
	Week 2	94.9 ± 0.26	90.04 ± 2.1	9.96 ± 2.1	1.5 ± 0.22[b]
	Week 3	93.95 ± 1.1	91.5 ± 0.72	8.47 ± 0.72	1.4 ± 0.011[b]
	Week 4	93.27 ± 0.31	92.97 ± 1.31	2.61 ± 0.89	2.61 ± 0.89[c]

Statistical analyses performed only for CP, NDF, ADF and ADL.
Values within treatments and columns which do not have a common superscript are different at $p<0.05$. No significant effects of date were found for values without superscripts.
[a] Only one pen of lambs for treatment CON remained during week 4.
[b] Standard deviations based on pens.
[c] All values are in percent.

was negligible seemed to be reasonable for both of these diets. The efficiency of use of energy for microbial protein production was assumed to be the same for CON and STR. This assumption was based on the findings of ARC (1984) that indicated relatively high production rates for microbial N for both all-roughage and mixed concentrate-roughage diets. Diet CON contained a significant amount of roughage; accordingly, estimated PER was identical for these two diets (Table 4). Given these assumptions, we consider our reported values to be conservative estimates.

It is now accepted that duodenal flux of undegraded protein from feed sources depends on ruminal outflow rate. Various methods for correcting laboratory values of protein degradability for outflow rate are in use. Hvelplund and Madsen (1990) proposed to calculate degradability from protein solubility in a buffer solution. Kabré and Petit (1994) used this equation in a modified form for the estimation of fish meal degradability in an experimental setting similar to this study. More commonly, degradability of protein supplements is estimated with the two-phase exponential decay process proposed by Ørskov and McDonald (1979). However, the latter model requires in situ degradation data for complete parameterization. These are frequently unavailable, thus to arrive at approximate estimates, least squares approximations (as a polynomial function of outflow rate) of degradability values estimated in various published applications of the Ørskov and McDonald (1979) were derived for three different degradability classes: fast (e.g., soybean meal), medium (e.g., fish meal), and slow (e.g., feather meal). The examples presented by Ørskov (1992) demonstrate that differences between slopes of the degradation curve of protein supplements exist and that they are typical for definable degradation or protein source classes. The resulting equations were:

$$P = 93.76 - 757.95{*}k + 3115.85{*}k^2 \text{ (fast)}$$

$$P = 69.87 - 630.43{*}k + 3367.75{*}k^2 \text{ (medium)}$$

$$P = 40.68 - 343.92{*}k + 1955.61{*}k^2 \text{ (slow)}$$

with P being the effective degradability of the protein supplement and k denoting the fractional rate of outflow from the rumen. For fish meal, the equation for medium degradability applies. An estimate of fractional rumen outflow rate k was estimated based on data presented by Elimam and Ørskov (1984b) for sheep. A linear relationship was derived to predict the outflow rate k of fish meal from the rumen as a function of level of intake in multiples of maintenance requirements (M): $k = 0.0017 + 0.01932 * M$. Although several studies on factors affecting outflow from the rumen have been reported (e.g., Mansbridge and Ørskov, 1980; Sriskandarajah et al., 1981; Lindberg, 1982; Elimam and Ørskov, 1984a,b,c; Elimam and Ørskov, 1985; Ørskov et al., 1988), they are largely incomparable to one another. Among the confounding effects are differences in species, breed, physiological status, and properties of the diet (roughage/concentrate proportions, particle size of the supplement and the base diet tested). More systematic research is required to develop robust equations for predicting k based on intake levels.

Fattet et al. (1984) calculated a value of 498 kJ/kg $LW^{0.75}$ for energy intake at maintenance for animals supplemented with protein. For the present calculations, a maintenance requirement of 490 kJ/kg $BW^{0.75}$ ME was assumed for the protein supplemented group. This value, however, may represent an overestimate due to the lower energy intake of treatment SFM compared to the protein-supplemented treatments of Fattet et al. (1984).

3. Results

3.1. Feed intake, refusals, and estimated nutrient uptake

Feed intake was influenced by highly significant treatment effects (Table 3). Average intake over time of the control diet was highly variable. Table 2 summarizes the proximate analysis of refusals. Effects of treatment, date, and the interaction of date by

Table 3
Average feed intake in kg day^{-1} (LSQ means)

Treatment	N	Intake
CON	71	1.85 ± 0.29^{a}
ST − FM[a]	125	0.41 ± 0.13^{b}
SFM	125	0.56 ± 0.13^{c}
STR	131	$0.44 \pm 0.11^{b,d}$

Values without a common superscript differ at $p < 0.05$.
[a] Denotes intake of the supplemented straw diet minus fish meal offered.

treatment were highly significant for CP content of refusals when all treatments were combined.

For treatment STR, a significant date effect was only observed for CP (highest for week 4). Date had a highly significant effect on CP content of the refusals for treatment SFM, with values being highest in the first week.

In vitro analysis of the straw samples yielded a true in vitro DM digestibility of 49.79% for the straw given without supplementation, and 52.61% for the straw given with supplementation. During the adjustment period, stratification of weights across treatments occurred; however, all response variables involving weight were corrected for weight differences. The four average weight data collected after the adjustment period were used to calculate energy intake per kg/$BW^{0.75}$. It was assumed that all molasses offered was consumed, even though minor quantities may have remained in the refusals. From proximate analysis of refusals and feeds it was calculated that in the first week, SFM lambs consumed on average 50.2% of the fish meal offered, and 89% of the straw in the diet. For subsequent weeks, it was assumed that all fish meal was consumed, based on conclusions from refusal analysis data (see above). Table 4 details estimated energy and protein consumption per kg/$BW^{0.75}$. For calculation of energy intake of CON animals, the energy density of the combined diet was estimated at 9.85 MJ/kg of feed as-fed; that is, effects of selective intake were not considered.

For the calculation of PER of the diet, microbial N was assumed to contain 80% amino acid N, and duodenal digestibility was assumed to be 80%. These coefficients were proposed by NRC (2000) for beef cattle. Efficiency of utilization of duodenal protein was assumed to be 80%. Thus, for N of microbial origin, 1 MJ of ME was calculated to provide approximately 0.67 g N at the tissue level. For N from escape protein origin, the same digestibility and efficiency of utilization values were assumed, so that 1 g of escape CP was estimated to provide approximately 0.1 g N at the tissue level (assuming CP = 6.25 * N). PER was calculated as total tissue-available grams of N per MJ of metabolizable energy intake per kg metabolic BW. Since no contribution to total tissue-available N from undegraded feed protein was considered for diets STR and CON, a constant PER results for these diets. Results are summarized in Table 4.

3.2. Metabolites. Glucose

Noteworthy are the high standard deviations observed in week 2 for glucose which tended to decrease over time (Fig. 1A). Treatment, collection

Table 4
Estimated daily average metabolizable energy intake and N flux

Diet	Week	Energy intake (kJ/kg $BW^{0.75}$)		Fractional outflow rate k[a]	P (%)[b]	N flux at tissue level (mg/kg $BW^{0.75}$)[c]	PER[d]
CON	1	902.9				659.1	0.73
	2	827.2				603.9	0.73
	3	763.2				557	0.73
	4	770.7				562.6	0.73
SFM	1	160.3	174.5[e]	0.0081	64.98	204	1.17
	2	217.2	234.7[e]	0.0101	63.85	341	1.45
	3	247.7	265.3[e]	0.0114	63.12	367	1.38
	4	283.3	310.9[e]	0.0129	62.30	406	1.31
STR	1	120.9	136.0[e]			88.4	0.65
	2	166.9	190.0[e]			121.8	0.65
	3	187.9	214.6[e]			137.2	0.65
	4	218.0	249.4[e]			150.1	0.65

[a] For fish meal supplement and calculated based on k = 0.0017 + 0.01392 * M.
[b] Estimated degradability of fish meal supplement.
[c] Sum of calculated escape protein and microbial protein; assuming a true digestibility of 0.85 and availability of 0.8 for escape protein.
[d] N flux divided by energy intake; energy intake is based on in vitro digestibility. Since N-flux is calculated on the basis of ME intake without consideration of undegraded protein flow, constant PER values ensue for diets CON and STR.
[e] Calculated based on in vitro digestibilities.

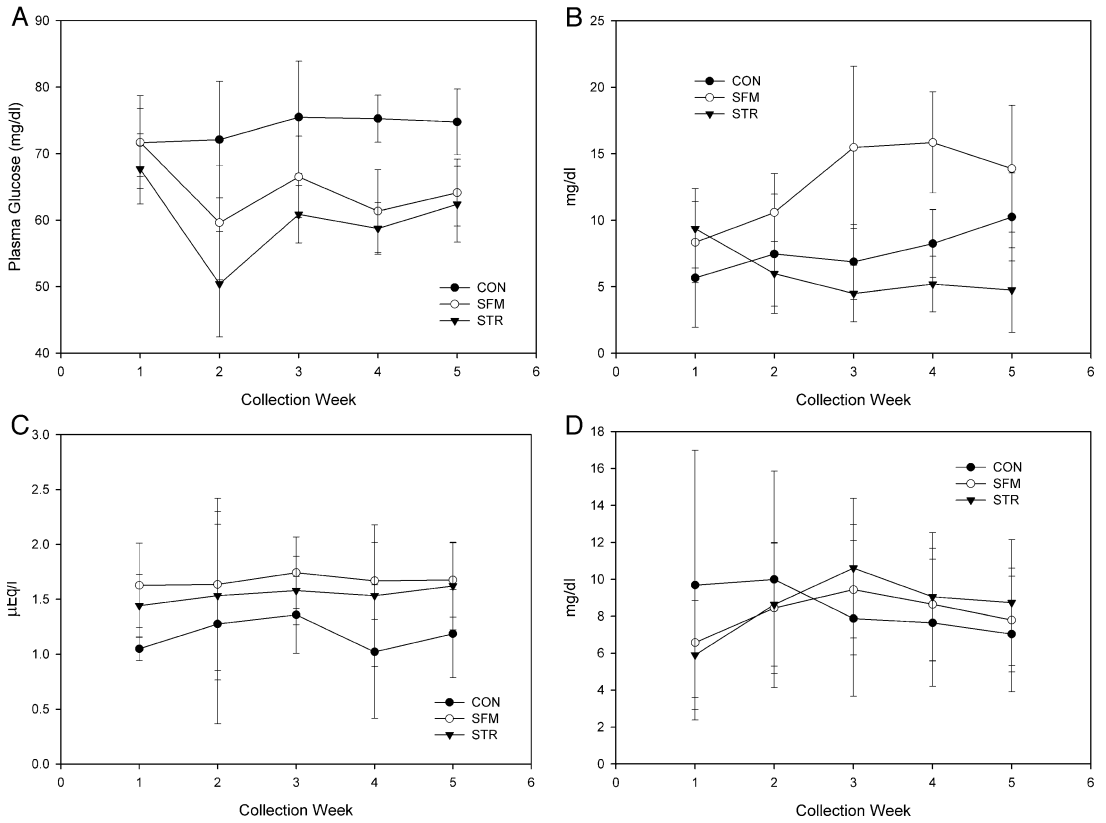

Fig. 1. Plasma concentrations of glucose (Panel A), urea (Panel B), NEFA (Panel C) and BHBA (Panel D) in lambs receiving the three diets CON=concentrate diet ad libitum, STR=straw ad libitum, SFM=straw ad libitum plus 150 g/d fish meal, over the duration of study.

week, and their interaction were all highly significant (Table 5). The contrast between treatments SFM and STR for the difference between first and last collection week confirmed that the glucose curves for the two treatments were essentially parallel (Tables 6 and 7).

3.3. Blood urea nitrogen (BUN)

Treatment effects on plasma BUN levels are presented in Fig. 1B. The increase in BUN concentrations for treatment SFM confirms results obtained by the refusal analysis. Treatment, collection week,

Table 5
Metabolite statistics

Effects			Contrasts			
Metabolite	Treatment	Collection date	CON vs. SFM		SFM vs. STR	
			Estimate	*p*-value	Estimate	*p*-value
Ketone bodies	Ns	Ns	1.55	Ns	−0.064	Ns
BUN	<0.0001	0.0014	−5.04	<0.0001	6.91	<0.0001
Glucose	<0.0001	<0.0001	8.91	<0.0001	5.33	0.0002
NEFA	<0.0001	<0.0001	−0.37	0.0109	0.13	Ns

Table 6
Contrasting specific treatment effects on the difference of metabolite levels between first and last collection date

Difference between first and last collection date			
Metabolite	Contrast	Estimate	*p*-value
Ketone bodies	CON vs. SFM	−0.209	Ns
	SFM vs. STR	0.313	Ns
BUN	CON vs. SFM	0.5401	Ns
	SFM vs. STR	Non-estimable	
Glucose	CON vs. SFM	−10.572	0.0004
	SFM vs. STR	0.697	Ns
NEFA	SFM vs. STR	0.084	0.41

and time by collection week interaction effects were all highly significant (Table 5).

3.4. Non-esterified fatty acids (NEFA)

Substantial depot fat mobilisation is indicated by plasma NEFA levels for treatments STR and SFM (Fig. 1C), which are about 50% higher than values reported by Kabré and Petit (1994) for ewes in negative energy balance. Diet SFM produced the numerically highest levels. Treatment and collection week effects were significant (Table 5).

3.5. Beta-hydroxy-butyrate (BHBA)

By week 3, BHBA levels (Fig. 1D) among SFM and STR lambs tended to be higher than in CON lambs. However, all means had very high standard errors and a biological interpretation is not straightforward. Effects of treatment, collection week, and their interaction were significant ($p=0.0001$ for all factors).

Table 7
Summary of sliced effect contrasts for differences between treatments SFM and STR for select metabolites

Collection date	Metabolite	Contrast	Estimate	*p*-value
1	Glucose	SFM–STR	4.0970	0.0132
2		SFM–STR	9.1446	0.0002
3		SFM–STR	6.2062	0.0015
4		SFM–STR	3.4000	0.0798
5		SFM–STR	3.8098	0.0206
1	BUN	SFM–STR	−1.0205	0.2804
2		SFM–STR	4.5469	<0.0001
3		SFM–STR	10.9205	<0.0001
4		SFM–STR	9.4996	<0.0001
5		SFM–STR	10.5816	<0.0001
1	BHBA	SFM–STR	0.6783	0.5785
2		SFM–STR	−0.2018	0.8625
3		SFM–STR	0.3654	0.7699
4		SFM–STR	0.03311	0.9752
5		SFM–STR	−1.1946	0.2705

Table 8a
Hormones: LSQ means

Hormone	Treatment	LSQ means (ng/ml)
IGF-1	CON	168.25
	SFM	135.38
	STR	101.92
GH	CON	3.75
	SFM	2.13
	STR	4.35
Leptin	CON	11.26
	SFM	3.78
	STR	3.39

3.6. Hormones. Leptin

Effects of treatment and time, and their interaction were highly significant (Table 8a, Fig. 2). There was no significant difference between treatments SFM and STR in the difference between leptin level at the first and at the last collection date (Fig. 2, Table 8b).

3.7. Growth hormone

Levels of GH exhibited a very high coefficient of variation (Fig. 3). Treatment and collection date

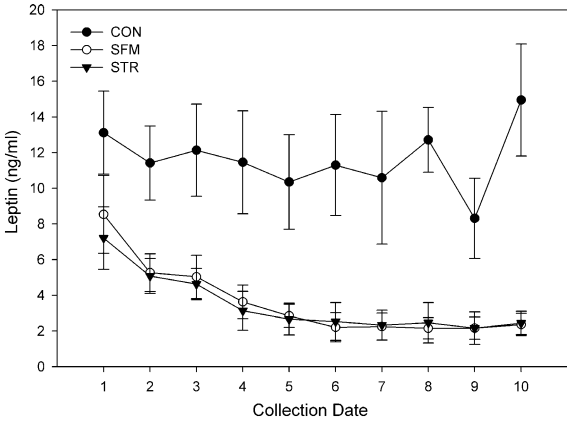

Fig. 2. Plasma concentrations of leptin (ng/ml) in lambs receiving the three diets CON=concentrate diet ad libitum, STR=straw ad libitum, SFM=straw ad libitum plus 150 g/d fish meal, over the duration of study.

Table 8b
Contrasting specific treatment effects on the difference of metabolite levels between first and last collection date

Difference between first and last collection date			
Hormone	Contrast	Estimate	*p*-value
IGF-1	SFM vs. STR	−52.75	0.0034
GH	SFM vs. STR	1.32	0.0001
Leptin	CON vs. SFM	−0.554	<0.0001
	SFM vs. STR	0.0059	Ns

Note that estimates for GH and leptin were performed on log-transformed values.

effects were highly significant (Table 8a). Interestingly, there was no difference between treatments CON and SFM in GH levels (Table 9), but the difference between SFM and STR was highly significant.

3.8. IGF-1

The IGF-1 data (Fig. 4) illustrate distinct effects associated with treatment and collection date effects (Table 8a). Both contrasts of interest were highly significant (Table 9).

4. Discussion

4.1. Feed intake, refusals and estimated nutrient uptake

Intake of the experimental diets exhibited a steady upward trend (Fig. 5), similar to the observations by Vipond et al. (1989). Absolute straw intake levels were also comparable to the results obtained by these workers. However, fish meal supplementation tended to decrease intake of the straw–molasses diet, although the difference between ST − FM (SFM minus fish meal) and STR was not significant ($p=0.074$). Fattet et al. (1984) also did not find a significant difference in straw intake between unsupplemented and supplemented diets. Thus, protein supplementation did not enhance intake of the straw diet, suggesting an intake limitation caused by energy deficit of the diet. Vipond et al. (1989) reported the opposite effect but did not indicate significance of the difference. There was no significant difference in CP content of refusals between weeks 2, 3, and 4. Vipond et al. (1989) reported a very rapid acceptance of the fish meal supplementation by lambs in their experiment; this was not the scenario in our study.

The difference between in vitro digestibility of straw with and without N supplementation is comparable in magnitude to in vivo results obtained for similar diets by Fattet et al. (1984) who found 64% digestibility for NaOH-treated barley straw given without and 68% for straw given with fish meal supplementation. Ortigues et al. (1990) and Kabré and Petit (1994) also found that fish meal supplementation significantly increased digestibility of a medium quality roughage under restricted intake levels. However, our values are considerably greater than the 43% reported by NRC (1985) for barley straw. On the other hand, the CP values found in our analysis were much lower than the NRC (1985) value of 4% (Table 4).

Calculated energy uptake values for the supplemented group were between those estimated by Vipond et al. (1989) and Fattet et al. (1984). Vipond et al. (1989) based their estimate of energy uptake on a ME content of 6.8 MJ/kg straw DM; this is slightly greater than the NRC (1985) value of 6.6 MJ/kg. These workers calculated energy intakes of 107 and 170 kJ/$BW^{0.75}$ for the unsupplemented and supplemented groups, respectively. Fattet et al. (1984) reported energy intakes based on a calculated ME content of 8.48 MJ/kg DM for NaOH-treated straw without and 8.89 MJ/kg DM for straw with supplementation. Accord-

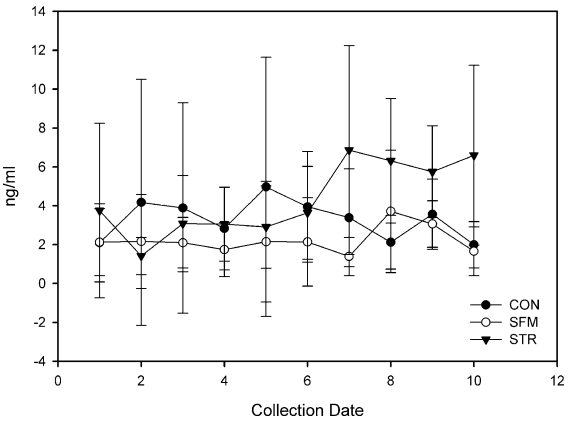

Fig. 3. Plasma concentrations of growth hormone (GH; ng/ml) in lambs receiving the three diets CON=concentrate diet ad libitum, STR=straw ad libitum, SFM=straw ad libitum plus 150 g/d fish meal, over the duration of study.

Table 9
Hormones: contrasts

Effects			Contrasts			
			CON vs. SFM		SFM vs. STR	
Hormone	Treatment	Collection date	Estimate	*p*-value	Estimate	*p*-value
IGF-1	<0.0001	<0.0001	32.87	0.0013	33.47	0.0002
GH	<0.0001	<0.0001	1.63	Ns	−2.22	<0.0001
Leptin	<0.0001	<0.0001	7.48	0.0001	0.39	Ns

ingly, the two straw–fish meal treatments in their experiments were estimated to have been consumed at 307 and 488 kJ/kg $BW^{0.75}$, respectively. Galbraith et al. (1997) estimated an energy uptake in their study of 188 kJ/kg $BW^{0.75}$ without reporting intake data.

Fattet et al. (1984) calculated an effective degradability of fish meal in their study of 0.416; when using their estimated level of energy intake of animals used for experimental determination of degradability, the equation relating digestibility to outflow rate presented above yields an effective degradability of 0.481. Vipond et al. (1989) estimated N flux derived from supplemental protein escaping ruminal digestion based on an assumed degradability of 0.3, which is much lower than estimated in the present study (approximately 0.6; Table 1) and seems not very probable in view of the prolonged rumen retention times likely with such low intake levels.

Energy intake and calculated N flux values (Table 4) are similar to those estimated by Vipond et al. (1989), but the difference in degradability estimates renders them not directly comparable. PER values are estimates based on several assumptions and therefore imprecise; however, they indicate that substantially higher fat mobilization in supplemented animals (compared to group STR) would have been required in order to use the additional protein for net tissue accretion.

4.2. Metabolites

Some of the additional protein may have been used in gluconeogenesis as suggested by the numerically higher plasma glucose levels for group SFM. Reilly and Ford (1971) reported a significant correlation between total glucose production rates and daily protein intake in sheep. Galbraith et al. (1997) did not find a difference in plasma glucose

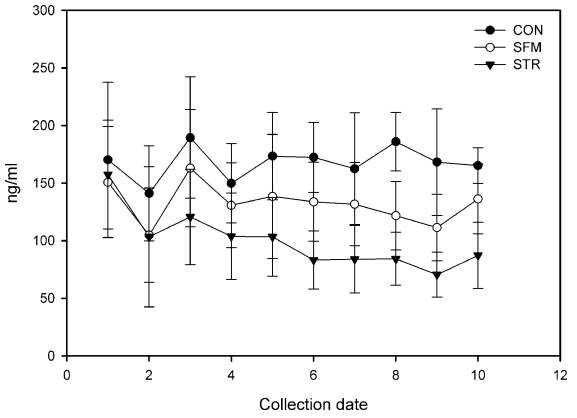

Fig. 4. Plasma concentrations of insulin like growth factor 1 (IGF-I; ng/ml) in lambs receiving the three diets CON=concentrate diet ad libitum, STR=straw ad libitum, SFM=straw ad libitum plus 150 g/d fish meal, over the duration of study.

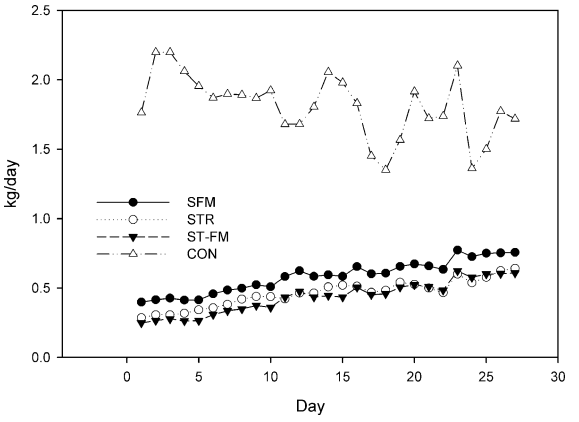

Fig. 5. Average feed intake among lambs receiving the three diets CON=concentrate diet ad libitum, STR=straw ad libitum, SFM=straw ad libitum plus 150 g/d fish meal, ST−FM=straw ad libitum minus fish meal over the duration of study.

levels between their treatments. Ortigues et al. (1990) found elevated levels of plasma glucose in heifers limit-fed a straw-based diet when supplemented with high levels of fish meal. However, when the base diet was fed at high levels, plasma glucose levels were highest at low levels of fish meal supplementation. Kabré and Petit (1994) reported mean glucose values of 61.4 mg/100 ml for unsupplemented, and 60.9 mg/100 ml for fish-meal supplemented ewes in negative energy balance, i.e. no potential net effect of fish meal on circulating glucose. These authors concluded from BUN and urine N values that the fish meal supplement was used as an energy source. Goetsch et al. (1994) did not observe an effect of a diet high in rumen undegradable protein on hepatic release of glucose in sheep of similar weight. The numerical value of liver net flux of glucose was slightly higher than a treatment high in rumen-degradable fibre in that study. In general, there are considerable difficulties in estimating dietary amino acid contributions to gluconeogenesis because labelling studies depend on the assumption of one homogenous body pool of the metabolite. Liver and kidney, for example, concentrate amino acids and this concentration may considerably distort quantitative estimates (Reilly and Ford, 1971; Waterlow et al., 1978).

As indicated by the BUN values, diet SFM clearly provided protein in excess, suggesting that N supplementation was not accompanied by a significant increase in mobilization of energy reserves. The negative quadratic effect of time was highly significant for treatment SFM. For treatment STR, a positive quadratic effect was also highly significant ($p=0.001$). This opposite pattern of BUN over time is noteworthy. In severely protein-restricted mammals, the rate of protein breakdown accelerates in very young, growing animals but not (in net terms) in adult animals (Waterlow et al., 1978). Thus, the very high BUN levels observed for SFM (compared to STR) point to dietary N origin, while the decreasing and stabilizing BUN levels in STR suggest an initially high breakdown (presumably due to rapid loss of splanchnic tissues) and a stage of maturity past high body growth rates. Thus, it is important to evaluate the effects of PER in growing animals relative to stage of maturity of experimental animals. Given the genetic heterogeneity of the animals in our experiment, the estimation of degree of maturity based on a breed specific standard growth curve would not have been possible; this situation appears to be the norm in the literature.

Ortigues et al. (1990) measured comparable effects of fish meal supplementation on BUN levels in heifers under a similar regimen. Galbraith et al. (1997) found a difference in BUN between fish meal supplemented and unsupplemented treatments similar to that observed in the present study. Lindberg and Jacobsson (1990) found significant effects on BUN levels of both energy and N concentration of intragastric infusates in lambs of comparable age and size. The highest level of BUN observed in the present study (week 4, treatment SFM) is approximately equivalent to values measured at an infusion of N at 1500 mg/kg $BW^{0.75}$ N given with 106 kcal/$BW^{0.75}$. Thus, a PER in our study of approximately 1.5 compares with PER of 2.2 in the study of Lindberg and Jacobsson (1990) at the corresponding observed BUN level. Interestingly, the highest level of plasma glucose observed in the present study (week 3, treatment CON) was comparable to the level of plasma glucose measured in lambs infused with 151 kcal/kg $BW^{0.75}$ and zero N in the study of Lindberg and Jacobsson (1990). These data provide evidence that a direct comparison between intragastric infusion experiments and conventional feeding may be misleading.

Galbraith et al. (1997) observed higher levels of NEFA for the unsupplemented treatment compared to fish meal supplementation. Kabré and Petit (1994) found NEFA levels of approximately 1.1 μEq/100 ml plasma for both supplemented and unsupplemented lambs, which would correspond to the values observed for treatment CON in the present experiment. However, the feed restriction period in that study was longer, and experimental lambs where in medium body condition at the beginning of that study.

On the other hand, BHBA levels over time do not suggest a simple interpretation (Fig. 1D). Table 7 summarizes sliced effect comparisons contrasting treatments SFM and STR for the 5 collection dates for metabolites. The sign of the difference SFM-STR changed, possibly indicating that, initially, size of fat depot was critical; then, as dependence upon fat mobilization increased in groups STR and SFM, differences in importance of energy sources appeared. The only week when SFM produced a numerically

higher average BHBA value than STR was the week during which fish meal consumption was about half of that for the other weeks. This does not suggest that higher N availability precipitated elevated levels of oxidation of depot fat FA. Interestingly, Vipond et al. (1989) could not find significant effects of nutritional treatments on plasma levels of BHBA; however, plasma levels of ketone bodies may not be an unequivocal indicator of degree of use of internal fat depots. In contrast to Vipond et al. (1989), we could not find a general trend of decreasing levels over time, with the highest value at the beginning of the experiment.

Collectively, the results of the metabolite assays suggest that the supplemental N in addition to a low energy diet did not increase utilization of fat stores for protein accretion. Clearly, these results differ from those obtained in intra-gastric infusion experiments and some conventional feeding trials. It is tempting to suggest that under conditions of below-maintenance energy intake in ruminants, the synchronization of supply of VFA and absorbable N at least in part explains differences in plasma metabolite levels between intra-gastric infusion nutrition and conventional feeding. Different synchronization of nutrient supply (or, equivalently, differences in PER effectively achieved) would be expected to lead to differences in the pattern of fat depot utilization and protein accretion. Thus, more detailed kinetic studies of ruminal nutrient production and ruminal and duodenal absorption, with the objective of elucidation of partitioning effects of PER, seem to be indicated. An analysis by Weston and Margan (1994) of calorimetry data collected on sheep fed chopped forage diets vs. concentrate diets indicated a consistently higher proportion of energy retained as protein for the forage diets at similar levels of CP in the diet. The results obtained here provide evidence supporting the conclusion by these workers that the relationship between CP absorbed in the intestine and protein balance is poor. Weston and Margan (1994) suggested that the composition of absorbed nutrients (which varies depending on synchronization of absorbable energy and protein supply) could have contributed to the observed partitioning differences. Differences in glucogenic substrate as they are likely in a comparison of concentrate vs. forage may be expected to influence hormonal regulation of partitioning by e.g. causing different insulin levels. Such a mechanism, however, may not explain the discrepancy between the results of the present study and those observed in intra-gastric infusion experiments. In addition, intra-gastric infusion regimes may cause changes in mass and function

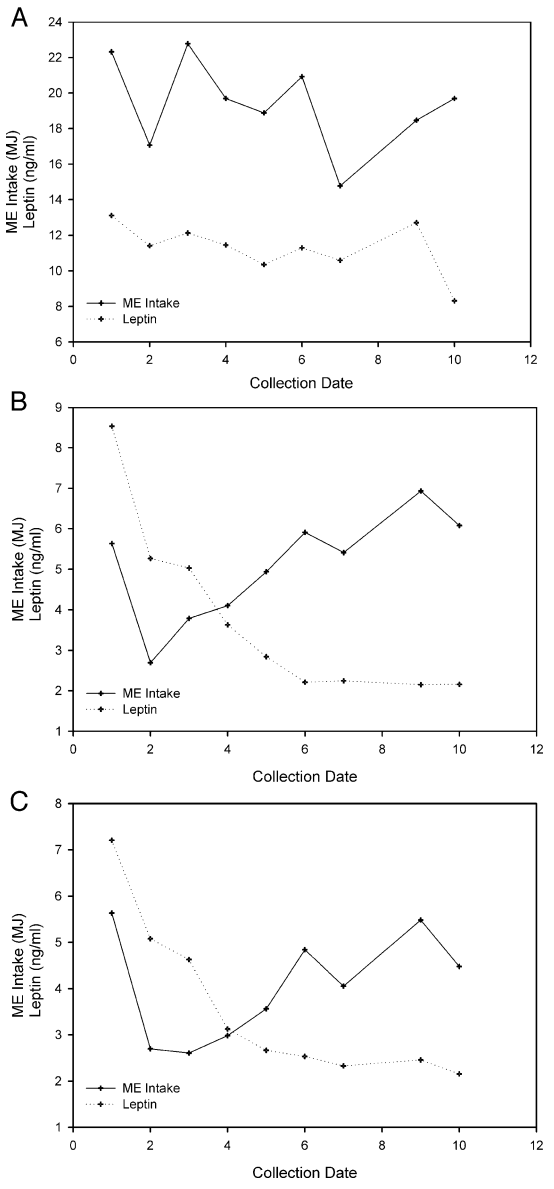

Fig. 6. Relationship between ME Intake and plasma concentrations of leptin (ng/ml) in lambs receiving the three diets CON=concentrate diet ad libitum (Panel A), STR=straw ad libitum (Panel B), SFM=straw ad libitum plus 150 g/d fish meal (Panel C), over the duration of study.

of metabolically active organs (GI tract). Such changes may explain in part the observed discrepancies, and require further research. However, a profound effect of duodenal tissue mass differences on whole-body protein utilization seems unlikely. In order to appropriately compare the effects of similar PER diets observed in studies using conventional feeding schemes, observations on lambs should be scaled to degree of maturity. The impetus for growth and thus energy demand in response to available N must be assumed to be highly dependent on potential growth rate. Without this information, definitive conclusions do not seem to be warranted.

4.3. Hormones

Fig. 6A–C, combining ME intake and leptin profiles, illustrate that only for treatment CON, the leptin curve tracked ME intake well (with the exception of one sampling date). The trends in the leptin curves for treatments SFM and STR (lambs in negative energy balance) are very similar, and are clearly not related to acute ME intake. Presumably, they reflect the declining fat depots in these lambs. Thus, it is suggested that in lambs fed below requirements for maintenance, there is no acute tracking of ME intake by leptin. The difference in patterns between lambs in positive and negative energy balance could be interpreted as circulating leptin levels having two 'components': one closely related to fat mass, and the other to current ME intake. The second component seems to cease being visible in lambs in negative energy balance. This may be attributed to the catabolisation of the tissue primarily responsible for leptin synthesis.

Table 10 summarizes the results of the sliced effect comparison and aids in interpreting Fig. 2. Since the values for treatment SFM tended to be numerically higher than those measured under treatment STR, an accelerated fat breakdown is not suggested by the data. Previous work reported relatively strong correlations between leptin levels and body fat mass in mature sheep (Delavaud et al., 2000), and growing cattle (Ehrhardt et al., 2000). Overall, our values are comparable with those reported by Delavaud et al. (2000) who used sheep fed at different levels of energy supply, although the values for our treatment CON are higher than the levels reported for high-

Table 10
Growth hormone, IGF-1 and leptin: selected sliced effect contrasts for collection dates

Date	Contrast	GH		IGF-1		Leptin	
		Estimate	*p*-value	Estimate	*p*-value	Estimate	*p*-value
1	CON–SFM	−0.09929	Ns	16.9863	Ns	0.2020	<0.0001
2	CON–SFM	0.2997	Ns	35.1576	0.0248	0.3379	<0.0001
3	CON–SFM	0.2122	Ns	29.7602	0.0593	0.3714	<0.0001
4	CON–SFM	0.3794	Ns	18.8227	Ns	0.4957	<0.0001
5	CON–SFM	0.6069	0.0328	31.2779	0.0377	0.5363	<0.0001
6	CON–SFM	0.7305	0.0123	33.2889	0.0015	0.6909	<0.0001
7	CON–SFM	1.0752	0.0010	33.2889	Ns	0.6069	<0.0001
8	CON–SFM	−0.5371	0.0433	62.0852	<0.0001	0.7343	<0.0001
9	CON–SFM	0.1086	Ns	52.6172	0.0003	0.4491	<0.0001
10	CON–SFM	0.1052	Ns	25.6194	Ns	0.7562	<0.0001
1	SFM–STR	−0.2349	Ns	−2.5020	Ns	0.2801	<0.0001
2	SFM–STR	0.2178	Ns	0.4007	Ns	0.3565	<0.0001
3	SFM–STR	−0.4094	Ns	39.5936	0.0060	0.4153	<0.0001
4	SFM–STR	−0.6087	0.0020	26.6644	0.0125	0.5727	<0.0001
5	SFM–STR	−0.5667	0.0216	33.9890	0.0101	0.5888	<0.0001
6	SFM–STR	−0.7439	0.0033	51.0969	<0.0001	0.6547	<0.0001
7	SFM–STR	−1.5750	<0.0001	49.7822	0.0002	0.6290	<0.0001
8	SFM–STR	−0.8064	0.0005	34.8547	0.0002	0.7277	<0.0001
9	SFM–STR	−0.7318	0.0003	50.5518	<0.0001	0.5277	<0.0001
10	SFM–STR	−1.5564	<0.0001	50.2523	<0.0001	0.7756	<0.0001

Note that GH and leptin estimates were performed on log-transformed values.

energy ad libitum fed animals in the study of Delavaud et al. (2000).

Under energy deprivation, the GH/IGF-1 axis is uncoupled (Breier and Sauerwein, 1995) and our results clearly illustrate this effect. IGF-1 levels were lowest for animals in diet STR, whereas the same animals, as expected, had the highest, continuously increasing GH levels (Fig. 3). On the other hand, the differences between CON and SFM in levels of GH were not significant for most collection dates (data not shown, but see Fig. 3). This seems to suggest an effect of the fish meal supplementation on GH not explained by the relatively minor difference in energy concentration (see Table 4) between diets STR and SFM. We submit that protein supplementation played an important role in this phenomenon. Likewise, the sliced effect comparison results for IGF-1 (Table 10) suggest that the strongly significant SFM-STR contrasts that appear after the first week are caused by differences in protein intake. Taken together, the data for GH and IGF-1 suggest that protein supplementation produced a discernable effect on key hormones involved in growth and energy metabolism. However, while the levels of GH and IGF-1 appear to suggest a growth-promoting effect of high PER in treatment group SFM, a concomitant increased release of stored energy was not observed. Thus, the effects of energy deprivation could not be alleviated by higher available N leading to higher mobilisation of body energy reserves.

5. Conclusions and implications

Our results support the argument that dietary PER is not an appropriate indicator of the ability of diets to modify utilization of endogenous energy reserves in sheep. Differences between conventional feeding trials, such as ours, and intra-gastric infusion regimens exist, and further studies of the synchronization of nutrient flux are required to understand the reasons for these differences. PER effects should further be evaluated relative to degree of maturity of animals. However, this information will be difficult to obtain when working with commercial animals of nondescript genetic origin.

Given the high cost of protein supplements providing substantial levels of protein escaping ruminal digestion, the post hoc modification of body composition of feedlot lambs seems to be inferior to feeding regimes attempting to minimize excess fat deposition during normal growth. Likewise, our data suggest that livestock producers in extensive systems cannot be successful in preserving protein mass in meat animals by protein supplementation in times of energy deficit of the diet.

The effects of the dietary treatments on hormones involved in the regulation of metabolism, particularly leptin, illustrate the strong effects of nutritional management. GH and IGF-1 levels reflected the effects of dietary energy, but seem to have been influenced by N supply as well. High levels of circulating GH do not reflect high growth activity, but severe energy deficit. IGF-1 was highest under high energy supply, but N supplementation led to IGF-1 levels substantially elevated above those of animals with only a slightly lower energy supply. More research is needed to understand the effects of protein supply under energy deprivation on hormonal growth regulation.

To our knowledge, this is the first report of a lack of leptin response to changes in ME intake, such as observed for treatments SFM and STR. More research is required to elucidate the re-partitioning effects of leptin in animals differing in body composition and fed below maintenance.

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